

Review

# Varietal Aromas of Sauvignon Blanc: Impact of Oxidation and Antioxidants Used in Winemaking

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**Abstract:** Key varietal characteristics of Sauvignon Blanc, including the descriptors of ‘green’ and ‘tropical fruit’, are mostly attributed to methoxypyrazines and volatile thiols, while monoterpenes, higher alcohols, esters, fatty acids, and other volatile compounds also add complexity and fruity notes to the wines. During the winemaking and ageing period, oxidation decreases the concentrations of these compounds and diminishes the flavours derived from this aromatic grape variety. Therefore, antioxidants, such as sulfur dioxide, are commonly utilized in Sauvignon Blanc wine production for better preservation of those beneficial primary aromas. This review focuses on key varietal aromas in Sauvignon Blanc wine and how they are influenced by oxidation, and SO<sub>2</sub> alternatives, including ascorbic acid, glutathione, and glutathione-enriched inactivated dry yeasts, that can be used in winemaking as antioxidants.

**Keywords:** antioxidants; glutathione; glutathione-enriched inactivated dry yeasts; methoxypyrazines; oxidation; Sauvignon Blanc; thiols



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## 1. Introduction

Sauvignon Blanc is one of the most popular white wines around the world. Originating from the Loire Valley of France, Sauvignon Blanc was considered a wild weed before winemakers started to turn grapes into wine hence the name Sauvignon Blanc came from the meaning of ‘wild whites’ in French. Some of the best grape-growing regions for Sauvignon Blanc in the world are namely Loire Valley (France), Bordeaux (France), Marlborough (New Zealand), California (United States), and Casablanca (Chile). The flavours and styles of Sauvignon Blanc may vary by region due to different terroir and winemaking practices, but typically, Sauvignon Blanc wines are made in a dry, still, and light-bodied style with high acidity and aromatic characteristics dominated by grape-derived fruity flavours.

Sauvignon Blanc vines in New Zealand were first commercially planted in Marlborough in the mid-1970s and the wines have gained worldwide recognition since then. Until now, Sauvignon Blanc is the most widely planted grape variety in New Zealand, with over 26,000 hectares of vineyard land devoted to growing the grape. The majority of which is planted in Marlborough (23k ha), followed by Hawke's Bay (1k ha) and Nelson (0.6k ha) [1]. Sauvignon Blanc accounts for 72% of New Zealand's wine production and makes up 86% of total wine exportation from New Zealand. The advantages of New Zealand's growing environment, including cool climate, low rainfall, long sunshine hours, large diurnal temperatures, and mixed soil types, aid in reaching the freshness and crispiness of Sauvignon Blanc grapes. Reductive winemaking, which aims at minimizing oxygen exposure during vinification, is commonly adopted for Sauvignon Blanc to reduce the loss of primary aromas and limit the development of oxidative characters.

## 2. Important Aroma Compounds in Sauvignon Blanc

Sauvignon Blanc is an aromatic variety typically having pronounced aromas of ‘green’ and ‘tropical’ characters. Green characters, including leafy, herbaceous, and grassy notes,

are generally related to a specific group of volatile compounds, methoxypyrazines, while tropical characters with aromas of gooseberry, grapefruit, and passion fruit are attributed to volatile thiols. Despite the varietal aromas of Sauvignon Blanc being dominated by methoxypyrazines and volatile thiols (Table 1), other aroma compounds (Table 2) derived from sugar and amino acid metabolisms during alcoholic fermentation, such as higher alcohols, fatty acids, esters, and to a lesser content, acetaldehyde, play a supportive role [2]. These volatile metabolites enhance wine complexity and impart fruity aromas that are not varietal specific to Sauvignon Blanc [3,4].

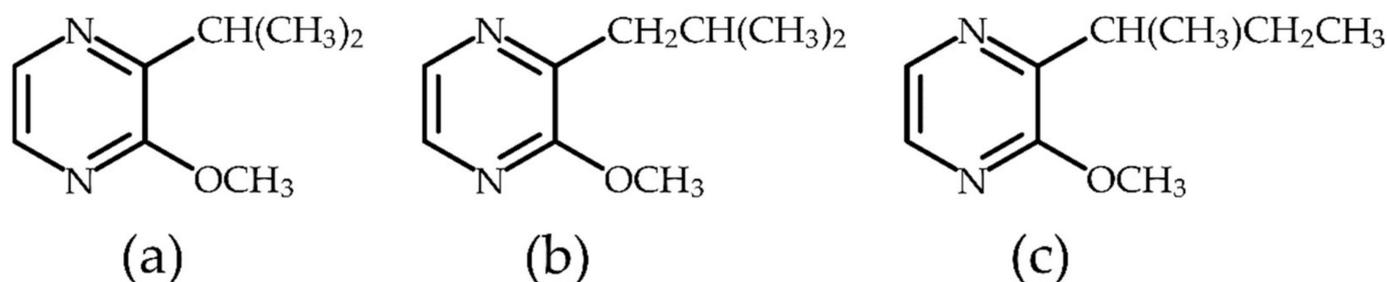
**Table 1.** Key varietal compounds in Sauvignon Blanc wines.

Compound	Sensory Description	Concentrations in Wine (ng/L)	Sensory Threshold (ng/L)	
Methoxypyrazines	2-methoxy-3-isobutylpyrazine (IBMP)	Asparagus, capsicum	0.4–56.3 [5,6]	2 [7] <sup>1</sup>
	2-methoxy-3-isopropylpyrazine (IPMP)	Earth, leaf	<0.03–13.7 [5,6,8]	2 [7] <sup>1</sup>
	2-methoxy-3-sec-butylpyrazine (SBMP)	Earth, leaf	<0.03–11.2 [5,9]	2 [7] <sup>1</sup>
Volatile thiols	4-mercapto-4-methylpentan-2-one (4MMP)	Box tree, passion fruit, black currant bud	4–40 [8,10]	0.8 [11] <sup>2</sup>
	3-mercaptohexan-1-ol (3MH)	Passion fruit, grapefruit, gooseberry, guava	200–18,000 [8,10]	60 [11] <sup>2</sup>
	3-mercaptohexyl acetate (3MHA)	Passion fruit, box tree	0–2500 [8,10]	4.2 [11] <sup>2</sup>
	Benzenemethanethiol (BMT)	Mineral, flint, smoke, burnt wood	10–15 [12]	0.3 [12] <sup>2</sup>

<sup>1</sup> determined in water; <sup>2</sup> determined in model wine.

### 2.1. Methoxypyrazines

Methoxypyrazines are commonly associated with the ‘green’ character in Sauvignon Blanc must and wine [13]. Methoxypyrazines are nitrogen-containing heterocyclic substances (Figure 1) biosynthesised as the secondary metabolites of amino acids, which are considered grape-derived aroma compounds [14]. Three methoxypyrazines in Sauvignon Blanc have very low perceptive thresholds in wine with general concentrations well above these threshold values [15].



**Figure 1.** Methoxypyrazines in Sauvignon Blanc grapes and wine. (a) 2-methoxy-3-isopropylpyrazine, IPMP; (b) 2-methoxy-3-isobutylpyrazine, IBMP; (c) 2-methoxy-3-sec-butylpyrazine, SBMP.

The 2-methoxy-3-isobutylpyrazine (IBMP) with asparagus or capsicum-like aromas has the highest concentration recorded at 307 ng/L in grapes and 56.3 ng/L in wines, and thus it is considered the main contributor to the green character of Sauvignon Blanc wine [13]. However, the correlation between IBMP and the ‘capsicum’ attribute is found to

be surprisingly weak [8], the explanation behind this may lean on the masking effect of other volatile compounds, such as varietal thiols, C6-alcohols, and dimethyl sulfide [8,16]. In addition, the study indicated when IBMP, (Z)-3-hexenol, and 1-hexanol were added together, the aroma presented in wine was changed from a veggie and earthy character to a pepper odour nuance, which showed a synergistic interaction among these compounds [17].

Both 2-methoxy-3-isopropylpyrazine (IPMP) and 2-methoxy-3-sec-butylpyrazine (SBMP) mainly contribute to earthy and leafy notes, however, they make subtle contributions to the green character due to their low concentrations present in grapes and wine. The aroma imparted by pyrazines was also found to depend on the presence of other compounds. In terms of the concentrations of pyrazines, up to 48.7 ng/L and 13.7 ng/L of IPMP were found in grapes and wines, respectively, and to a lesser content, the highest concentration of SBMP was recorded at 11.2 ng/L in both grapes and wines [8].

Studies conducted on red wine grapes showed that IBMP begins to accumulate in berries 10 days after anthesis, and declines rapidly during the maturation afterwards [18,19]. Final concentration of IBMP is determined before véraison [20], while early basal removal from the fruiting zones was found to reduce IBMP accumulation effectively [21]. The concentration of methoxypyrazines also depends on the climate of growing regions as the accumulation and degradation of these compounds are sensitive to temperature and sunlight exposure [20,22,23].

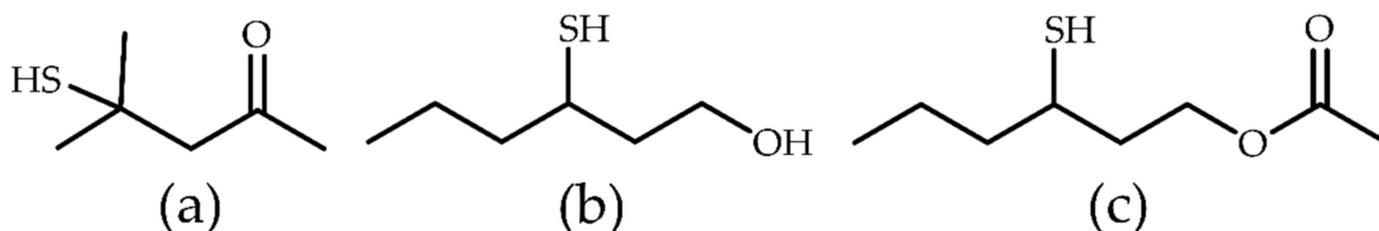
## 2.2. Volatile Thiols

Volatile sulfur compounds in wine can be divided into two classes based on the aromas perceived by humans, either negative or positive. On the one hand, some sulfur-containing compounds are responsible for off-flavours, such as rotten eggs ascribed to H<sub>2</sub>S produced by wine yeast [24,25], and cooked vegetables caused by thioacetic acid esters and mercaptans under low redox potential in wine [25]. On the other hand, certain sulfur-containing compounds can contribute to positive odours such as the notes of gooseberry, passion fruit, grapefruit, and guava [3]. These compounds considered to be the impact odorants in New Zealand's Sauvignon Blanc are referred to as volatile thiols or polyfunctional mercaptans [26]. Volatile thiols contain one or more sulfhydryl groups with additional functional groups such as ketones, alcohols, and esters in their molecules [3], and they can be perceived by the human nose at very low concentrations [27].

Three important volatile thiols, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA) (Figure 2), are key aroma compounds of New Zealand Sauvignon Blanc, i.e., imparting aromas of box tree and black currant bud contributed by 4MMP [28], and aromas of passionfruit and grapefruit contributed by 3MH and 3MHA [11]. The perceptive thresholds of 4MMP, 3MH, and 3MHA in model wine are 0.8 ng/L, 60 ng/L, and 4.2 ng/L [11], and their concentrations in Sauvignon Blanc wines from France and New Zealand are reported in the range of 4–40 ng/L, 200–18,000 ng/L, and 0–2500 ng/L, respectively [29]. If present at high concentrations, these compounds can emit sweaty aromas of cat urine character [30]. The biosynthesis of 3MHA is the result of the esterification of 3MH with acetic acid during fermentation, which is controlled by yeast ester-forming alcohol acetyltransferase encoded by the ATF1 gene [31]. The amount of 3MHA depends on the conversion rate differed in yeast strains [32], which is normally up to 10% of 3MH [33]. Previous studies have proposed three biogenesis pathways to explain the formation of 3MH and 4MMP in wine.

The first pathway involves cysteinylated precursors, S-3-(hexan-1-ol)-cysteine (Cys3MH) and S-3-(4-mercapto-4-methylpentan-2-one)-cysteine (Cys4MMP), which originate from grapes and are cleaved of the carbon-sulfur linkage by wine yeast through its beta-lyase activity during fermentation [33]. The second biogenesis pathway involves glutathionylated precursors, S-3-(hexan-1-ol)-glutathione (Glu-3MH) and S-3-(4-mercapto-4-methylpentan-2-one)-glutathione (Glu-4MMP), which also originate from grapes and act as the precursors of both volatile thiols, 3MH and 4MMP, respectively [34]. Apart from this, Glu-4MMP also acts as the precursor of Cys3MH deciphering part of the Cys3MH biogenesis pathway [35]. The

third biogenesis pathway involves the direct reaction of H<sub>2</sub>S and unsaturated C<sub>6</sub> compounds, such as (E)-2-hexenal and (E)-2-hexenol [36]. H<sub>2</sub>S can be produced by yeast metabolism of different sulfur sources, including elemental sulfur. The reduction of elemental sulfur by grape enzymes or other reducing agents, i.e., without the involvement of microorganisms, has also been suggested [37]. C<sub>6</sub> compounds are formed through the enzymatic breakdown of polyunsaturated fatty acids catalysed by lipoxygenase, hydroperoxide lyase and isomerase [38]. While the importance of this pathway remains to be determined, it has been demonstrated that suppling H<sub>2</sub>S to grape juice directly (Harsch paper again) or indirectly via elemental sulfur additions [37] invariably leads to an increase in 3MH and 3MHA concentrations in wine.



**Figure 2.** Volatile thiols found in Sauvignon Blanc wines. (a) 4-mercapto-4-methylpentan-2-one, 4MMP; (b) 3-mercaptohexan-1-ol, 3MH; (c) 3-mercaptohexyl acetate, 3MHA.

Apart from three varietal thiols that contributed mainly to tropical fruit aromas, there is another volatile thiol, benzenemethanethiol (BMT) that is worth mentioning. BMT was found to be responsible for some minerality characteristics, including flint, wet stones, and smoke in white wines [12,39], especially for a gun flint aroma presenting in Sauvignon Blanc wine [12]. However, the mineral character perceived in wine is considered a complex issue as it can be associated with various aspects of wine composition, including other aroma compounds [40], acidity [41], and reductive character [42]. The contribution of BMT to minerality in wine needs further investigation.

**Table 2.** Other volatile compounds in Sauvignon Blanc wines.

Compounds	Sensory Description	Concentrations in Wine	Sensory Threshold	
Terpenes	Linalool	floral, citrus	7.2–24.3 µg/L [43,44]	100 µg/L <sup>1</sup> [45]
	Geraniol	freshly cut grass	1.0–2.8 µg/L [46]	130 µg/L <sup>1</sup> [45]
Higher alcohols	2-phenylethanol (2-PE)	Rose, honey, spice	43.4–436 µg/L <sup>3</sup> [43,47]	200 mg/L <sup>2</sup> [48]
	Isoamyl alcohol	Solvent, whiskey, malt, burnt	80–300 mg/L [10,46]	60 mg/L <sup>2</sup> [48]
Esters	Isoamyl acetate	Banana, pear	2080–2880 µg/L <sup>3</sup> [43]	50 µg/L <sup>2</sup> [48]
	Ethyl hexanoate	Apple, banana, violets	999–2892 µg/L [46]	45 µg/L <sup>2</sup> [46]
	2-phenylethyl acetate (2-PEA)	Rose, fruity, honey	0.21 mg/L [49]	1.8 mg/L <sup>2</sup> [48]
Volatile fatty acids	Acetic acid	Vinegar	150–900 mg/L <sup>3</sup> [50]	1130 mg/L <sup>3</sup> [51]
Others	Acetaldehyde	Bruised apple, grass, nut, sherry	7–240 mg/L <sup>3</sup> [52]	100 mg/L <sup>3</sup> [53]
	Methionol	Asparagus, potato, tomato	529–728 µg/L [46]	500 µg/L <sup>2</sup> [54]
	2-furanmethanethiol	Roast coffee	0.42–0.44 ng/L [55]	0.4 ng/L <sup>2</sup> [55]
	Sotolon	Curry	n.d.–36 µg/L [56]	8 µg/L <sup>3</sup> [57]

<sup>1</sup> determined in Muscat Alexandre, Muscat Blanc wines; <sup>2</sup> determined in model wine; <sup>3</sup> determined in dry white wine.

### 2.3. Terpenes

Monoterpenes and their oxygen-containing derivatives, monoterpene alcohols, are C<sub>10</sub> compounds commonly found in grapes. Within more than 50 monoterpenes identified

in grapes, linalool, citronellol, geraniol, nerol, and  $\alpha$ -terpineol are the most abundant monoterpenes, especially in aromatic grape varieties, such as Muscat grapes, Riesling, and Gewürztraminer [58,59]. They contribute to floral (rose-like in particular), fruity, citrus, and perfume odours [60]. Even though the concentrations of terpenes are generally below the sensory threshold in Sauvignon Blanc, terpenes can still have a synergistic impact on the overall aromas present in wines [61].

Monoterpenes can be present in grapes in free form, polyhydroxylated form, or glycosidically bounded form [59]. Free forms of monoterpenes are odour-active compounds that include additional monoterpene ethyl esters and acetate esters present in wine. Polyhydroxylated forms of monoterpenes are free odourless polyols that make no direct contribution to wine aroma but can break down to release pleasant volatiles [59], i.e., diendiol can form hotrienol and nerol oxide (Williams, Strauss, & Wilson, 1980); (E)-3,7-dimethyl octa-2,5-dien-1,7-diol can form cis-rose oxide [62]. The majority of monoterpenes in grapes are glycosidically conjugated and are considered potential aroma compounds as they can be hydrolyzed into aroma-activated forms by acid or enzymatic hydrolysis during fermentation [63,64].

Monoterpene biosynthesis firstly involves the production of isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl pyrophosphate (DMAPP), through the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway catalysed by terpene synthases (TPSS) and monoterpeneol  $\beta$ -D-glucosyltransferases (GTs) in grape [65]. Then, IPP and DMAPP are condensed to form geranyl pyrophosphate (GPP) under the action of geranyl diphosphate synthase [66]. Finally, GPP is catalyzed by terpene synthases to form monoterpenes [67].

#### 2.4. Higher Alcohols

Among all the yeast-derived volatile compounds, higher alcohols and esters are the two most abundant groups in wines [68]. Higher alcohols, also known as fusel alcohols, are alcohols that have more than two carbon atoms, and are mostly derived from amino acid metabolism during fermentation via the Ehrlich pathway, which includes transamination, decarboxylation, and reduction. After the initial transamination, amino acids are transferred into  $\alpha$ -keto acids, which are then decarboxylated into fusel aldehydes and reduced into fusel alcohol [69]. Higher alcohols can be classified into aliphatic and aromatic alcohols based on amino acids that they are assimilated from [70]. Aliphatic alcohols are derived from linear- or branched-chain amino acids and have a strong solvent-like odour. Aromatic alcohols contain at least one benzene ring, which can enhance wine complexity and contribute positively to wine aromas when present at around 300 mg/L in white wines [71–73]. However, excessive higher alcohols (over 400 mg/L), including both aromatic and aliphatic alcohols, could contribute to a pungent smell and taste, and thus negatively affect the quality and character of wines [32,74]. The most important aromatic alcohol found in wines is 2-phenylethanol (2-PE), which is derived from phenylalanine and is associated with a rose-like aroma [75,76]. The 2-PE is naturally present in grapes at an undetectable concentration [49], however, certain yeast strains can enhance the production of 2-PE by increasing the production of its precursor, phenylalanine [77]. Apart from yeast strain selection, there are other factors during alcoholic fermentation that can affect the production of higher alcohols, such as fermentation temperature, juice clarity, oxygen levels, and amino acid composition [71,73]. Grape variety also has an impact on the concentration of higher alcohols, both general trends, and individuals. Overall, white wine varieties contain lower concentrations of higher alcohols than red wine varieties; individually, for example, more isoamyl alcohol is detected in Sauvignon Blanc than in Chardonnay [78].

#### 2.5. Esters

Esters are found in wine through the reactions between alcohols and fatty acids. Yeast-derived esters can be categorised into acetate esters and ethyl esters [30]. In white wines, acetate esters largely contribute to the floral and tropical-fruity character of the wines [79], and ethyl esters contribute to wine complexity with the hints of tree fruits aromas at low

concentrations but forming off-flavour like wax and honey at high concentrations [71]. High concentrations of esters in Sauvignon Blanc can be produced by selected yeast strains, which could have a masking effect over the green notes pertinent to methoxypyrazines [80]. The formation of acetate esters is mainly influenced by the yeast strains and the concentration of higher alcohols, while the formation of ethyl esters largely depends on the fermentation rate, which can be affected by the fermentation temperature and oxygen exposure [81]. Among these significant esters, the most critical esters identified in white wines that contribute to wine aromas are acetate esters of higher alcohols, such as isoamyl acetate, ethyl hexanoate and 2-phenylethyl acetate (2-PEA) [82]. Isoamyl acetate relates to banana and pear nuances, while ethyl hexanoate is associated with apple, banana, and violets notes. 2-PEA formed from the esterification of 2-PE contributes to the aromas of rose, fruity, and honey in wines [32]. Similar concentration ranges of most esters are found in Sauvignon Blanc to those in other white wine varieties except for a few compounds, such as ethyl hexanoate and ethyl isovalerate with higher concentrations, and ethyl isobutyrate with lower concentrations are quantified in Sauvignon Blanc [46].

### 2.6. Volatile Fatty Acids

Volatile fatty acids in wines are referred to a group of short- or medium-chain organic acids, including those responsible for volatile acidity (VA). The concentrations of volatile acids normally range from 500 to 1000 mg/L, and about 90% of them are acetic acid [50,83]. Acetic acid possesses a vinegar-like aroma when the concentration is near its perception threshold (0.7–1.1 g/L) [73]. Excessive production of acetic acid is usually associated with microbial spoilage of *Acetobacter* and *Gluconobacter* [84].

Medium-chain fatty acids in wine, such as hexanoic, octanoic, and decanoic fatty acids, are found to play an essential role in the fruity notes of wine [3]. These fatty acids are believed to be produced by yeast as intermediates in the biosynthesis of long-chain fatty acids [73]. The biosynthesis starts with the formation of acetyl coenzyme A (acetyl-CoA) from the oxidative decarboxylation of pyruvic acid, followed by the action of the fatty acid synthase complex [85].

The factors affecting the concentrations of volatile fatty acids include yeast strains, fermentation temperature, and juice nutrients. As for higher alcohols, the concentration of volatile fatty acids in wine is also depending on grape varieties, and differences in the concentrations of several volatile acids have been reported between Sauvignon Blanc and Chardonnay wines. Significantly higher concentrations of acetic acid, decanoic acid, octanoic acid, and hexanoic acid are found in Sauvignon Blanc than in Chardonnay wines [86].

### 2.7. Other Volatile Compounds

Volatile aldehydes contribute to different flavours, including notes of citrus, apple, grassy, pungent and nutty depending on the chemical structures [24,87], and they are of importance to wine aroma and flavours as they have low sensory thresholds. Volatile aldehydes are the intermediates in the Ehrlich pathway involving the formation of higher alcohols from amino acids and sugar [69], therefore, conditions favour the production of higher alcohols and also favour the formation of aldehydes [88]. Main aldehydes found in wine include acetaldehyde, 3-(methylthio)-propionaldehyde (methional), and phenylacetaldehyde, which are mainly generated from the oxidation of corresponding alcohols [89,90].

Acetaldehyde formed predominantly from the oxidation of ethanol via the coupled auto-oxidation of certain phenolic compounds [91], accounting for more than 90% of total aldehydes contents in wines [70]. The concentration of acetaldehyde in dry white wines ranges from 7 to 240 mg/L [52]. Methional and phenylacetaldehyde have also been found to occur via the Strecker reaction in which dicarbonyl compounds react with amino acids, methionine, and phenylalanine, respectively [89,90]. They have perception thresholds of 0.5 µg/L and 1 µg/L [89,92] with the concentrations in young Sauvignon Blanc wine at bottling below 0.5 and 5 µg/L, respectively [93].

Sauvignon Blanc wines are normally produced in a crisp and acidic way with fruit-driven aromas, however, oak-related aroma compounds can still be found in Sauvignon Blanc wines matured in barrels. For example, 2-furanmethanethiol contributing to roast coffee aroma is found in barrel-aged Sauvignon Blanc as the biogenesis of furanthiol derivatives requires furfural extracted from oak [94]. Sotolon (4,5-dimethyl-3-hydroxy-2(5)H-furanone), a volatile furanone with an intense curry odour that causes a detrimental effect on dry white wines [57,95], is also found in barrel-aged wines. Its concentration even exceeds the perceptive thresholds (8 µg/L) in Sauvignon Blanc wines aged in barrels without yeast lees, as the formation of sotolon in wines involves oxygen which can be easily reached without the protection from yeast lees during the ageing period [57].

### 3. Oxidation of Aroma Compounds in Sauvignon Blanc

Grape must and wine are inevitably exposed to oxygen during winemaking, and the amount of oxygen dissolved in wine can be up to 8 mg/L at cellar temperatures and air pressure [96]. Either insufficient or excessive amounts of oxygen in wine can lead to negative effects on wine quality. Too little oxygen exposure is associated with the formation of reductive characters in wine, while too much oxygen exposure causes the oxidation of both grape- and fermentation-derived aroma compounds, the development of undesirable oxidative odours, and the acceleration of colour browning [97,98]. Oxidation is detrimental for white wine, especially for Sauvignon Blanc wine, which is typically pale in colour and with pronounced fresh and fruity aromas.

#### 3.1. Oxidation Mechanism for Volatile Thiols

The cysteinylated and glutathionylated precursors of volatile thiols cannot be directly oxidised during fermentation, as the C–S bond in the precursors is quite stable towards oxidation [27]. However, after alcoholic fermentation, volatile thiols have been released and their concentrations in wine can diminish largely by various mechanisms, including oxidation and nucleophilic addition, as they are highly reactive and chemically unstable [3]. First, volatile thiols can be easily oxidised into the corresponding disulfides in the presence of oxygen and trace amounts of metal ions [99,100]. Second, these compounds are nucleophilic and can undergo substitution reactions with polymeric phenolic compounds, with detailed results proposed by Nicolantonaki et al. that (–)-epicatechin was more reactive with thiols than (+)-catechin and the presence of Fe (III) favoured the oxidation reactions [101]. Third, these compounds can also take part in Michael addition reaction with phenolic oxidation products, such as *o*-quinones [102].

In the third mechanism, phenolic compounds react directly with RSO (reactive species of oxygen) which are activated oxygen species formed by the reduction of O<sub>2</sub> in the presence of metals, such as Fe<sup>2+</sup> [101]. Two types of RSO are found related to phenolic oxidation, free superoxide (O<sub>2</sub>•<sup>–</sup>) and peroxide (O<sub>2</sub><sup>2–</sup>) radicals, while peroxide (O<sub>2</sub><sup>2–</sup>) radicals are present in hydrogen peroxide form at wine pH [103,104]. Then, the oxidation of phenolic compounds undergoes a series of chemical transformations and forms semiquinone radicals and quinones, which are both catechol derivatives [101]. The formation of these catechol derivatives initiates in two pathways, oxidation by the intermediates from the reduction of oxygen to hydrogen peroxide [103,105] and direct oxidation by Fe<sup>3+</sup> under acid conditions [101]. The acid conditions provided by wine preferentially go through the pathway of direct oxidation by Fe<sup>3+</sup> to reduce the formal reduction potential of the Fe<sup>3+</sup>/Fe<sup>2+</sup> couple caused by RSO formation and further facilitate the redox cycling [103]. Finally, the phenol oxidation products, which are electrophilic, unstable, and highly reactive, can react with other compounds with low redox potentials, such as phenolic molecules, SO<sub>2</sub>, and thiol-containing compounds, including glutathione and amino acids [106].

Quinones can react directly with nucleophilic thiols by a Michael addition reaction [104,107], and the thiols are degraded to an odourless form causing a loss of fruit character in the wine [108]. Specifically, the *o*-quinones derived from caftaric acid or (+)-catechin reacting with 3MH are most likely to be responsible for the loss of 3MH in wine under oxidative conditions [109].

### 3.2. Oxidation during Winemaking

Winemaking practices from harvesting, grape crushing, skin contact, and pressing, to alcoholic fermentation can input various amounts of oxygen into the musts. Winemaking decisions such as different harvesting methods, skin contact time, and pressing pressure applied should be made into consideration when producing Sauvignon Blanc wines, as the precursor content and the extraction rate in the juice leading to different concentrations of volatile compounds in the corresponding wines will be affected [110].

During the winemaking process, a high oxygen consumption rate, related to enzymatic activity, occurs with grape crushing [111]. A study demonstrated that adding SO<sub>2</sub> and ascorbic acids at crushing produces more pleasant wines with richer and more delicate aromas [111]. Skin contact and pressing under nitrogen increase the concentrations of glutathione (a natural antioxidant present in grapes) in Sauvignon Blanc wine [112,113]. Although longer skin contact time increases Cys3MH in the juice and larger pressure applied during pressing aids the release of volatile compounds [114], the oxidative potential of the juice also increases, which affects the concentrations of phenolic compounds dramatically, leading to decreased concentrations of 3MH and 3MHA in the resulting Sauvignon Blanc wines [115].

Methoxy-pyrazines are less prone to oxidation during winemaking. The concentration of IPMP was found to decrease at the stage of juice settling [15] and increase during the first day of maceration as methoxy-pyrazines are mainly found in the skins, seeds, and stems of the grapes and these compounds are extracted from grape musts to juice [116]. After racking, methoxy-pyrazines in Sauvignon Blanc wines level off regardless of the O<sub>2</sub>/SO<sub>2</sub> additions at the juice stage [117,118].

The effects of the fermentation vessels on the chemical, physical, and sensory attributes of Sauvignon Blanc wines were compared, showing that different materials of the vessels affect the carbon dioxide and the oxygen dissolved in juice. As a result, juice fermented in stainless steel tanks with less oxygen dissolved produced higher concentrations of volatile fatty acids and esters, while juice in polyethylene tanks and clay jar received lower levels of these volatile compounds [113].

### 3.3. Oxidation during Ageing

Despite oak barrels have an antioxidant capacity that influences the oxidation potential of the wine and a positive correlation was demonstrated between the oxidative stability of the wine and barrel ageing [119], barrel maturation is not a common winemaking practice to produce Sauvignon Blanc wines, except for a specific style known as Fumé Blanc, developed in the United States. However, some Sauvignon Blanc wines will go through bottle ageing due to transportation or storage required from time to time, and oxidation during this ageing period can affect the aroma compounds significantly.

Closures with consistently low oxygen transfer rates, such as screw caps and micro-agglomerated corks, were found to be effective in detaining the oxidation of Sauvignon Blanc wine during long-term ageing [120], the oxidative status of the bottle-ageing wine between closures can be visually indicated by the degree of browning [121].

The concentration of methoxy-pyrazines during ageing was studied on Riesling and Cabernet Franc wine regarding the closure effects. The result showed that the concentration of IBMP decreased by 30% after 12 months of bottle-ageing, with the highest concentration retained in wines bottled with corks, followed by screw caps, synthetic corks, and Tetrapak cartons decrease the most after 18 months of bottle-ageing [122,123]. However, most studies suggested that bottle ageing had no measurable effect on the concentrations of methoxy-pyrazines in Sauvignon Blanc wine [124], and they remain stable even under progressive oxidative storage [118].

Volatile thiols are particularly prone to oxidation during bottle-ageing, and they can easily oxidise to corresponding disulfides [100]. During the bottle-ageing period of Sauvignon Blanc wines, 3MHA is considered to be the least stable one that decreases steadily throughout the first year of bottle-ageing [100] while 3MH first level off then

increases after 3-month ageing, and the hydrolysis of 3MHA to 3MH is pointed to be the reason [125]. The concentrations of 3MH decrease after 6-month bottle ageing and continue declining as the ageing periods extended [93].

Closure and packaging options affecting the amounts of oxygen transferred through the bottles are also important to the concentrations of volatile thiols preserved in wines. 3MH was found to be better preserved in wines ageing in glass bottles compared to other configurations [93], while wines sealed with screw caps and bottle ampules displayed higher contents of volatile thiols compared to wines bottled using closure with higher permeability to oxygen [52].

The evolutions of other volatile compounds during ageing are mostly illustrated in other white wines as they are not specific to Sauvignon Blanc and are less prominent compared to methoxypyrazines and volatile thiols. Monoterpenes, such as linalool and  $\alpha$ -terpineol, were found to decrease during storage [126,127], while increases of monoterpene alcohol derivatives, such as linalool oxides, nerol oxide, and hotrienol, were observed during ageing [128].

Wine aromas change drastically during the ageing period, in which the concentrations of yeast-derived compounds are significantly affected by oxygen exposure. Higher alcohols are supposed to decrease as they can be oxidised to form aldehydes during ageing [129], however, the concentrations of higher alcohols were reported to be stable [130,131] while hexanol was found to increase probably from the oxidation of linoleic and linolenic acids [132]. Ester concentrations decrease during bottle ageing due to chemical hydrolysis [126,127] or ester interactions with *o*-quinones or oxidation caused by a direct attack by hydroxyl radicals [97,133]. On the other hand, the concentrations of fatty acids are supposed to increase due to the hydrolysis of the corresponding ethyl ester, however, some compounds were reported to increase while others decrease or remain stable during ageing [122,134].

Oxidative aromas of white wines described as honey, woody, and cooked vegetables are mainly attributed to aldehydes, lactones, and acetals [95,97]. Under extreme storage conditions, such as higher dissolved oxygen concentrations and higher storage temperatures [95], the concentrations of methional and phenylacetaldehyde in dry white wines were found to increase significantly. Packaging materials with different gas exchange rates can influence the wine even under a short period of ageing. Higher concentrations of methional, phenylacetaldehyde, and sotolon were found in wines ageing in Bag in Box<sup>®</sup> and PET (polyethylene terephthalate) monolayer bottles compared to wines ageing in PET multi-layer bottles and glass bottles as oxygen is allowed to permeate and the wines are easily oxidised [93].

#### 4. Antioxidants Used in Wine Production

Sulfur dioxide has been used as the main antioxidant in wine production at various winemaking stages, even though it occurs naturally in the wines as a by-product of yeast metabolism during fermentation [135]. Wines without any SO<sub>2</sub> additions will still have 10–20 mg/L of total SO<sub>2</sub> at the end of the alcoholic fermentation originating from yeast metabolism of amino acids [136]. The majority of SO<sub>2</sub> is mainly added in the form of potassium bisulfite, with typical concentration of free SO<sub>2</sub> ranging from 20 to 40 mg/L to protect juice or wine from oxidation, as well as, to keep the molecular SO<sub>2</sub> concentration below its sensory threshold of 2 mg/L.

Volatile thiols could be well preserved in red wines and model wines by the use of SO<sub>2</sub> under oxidative winemaking conditions in the presence of polyphenols [101,102,137]. The maximum amounts of 3MH and 3MHA were found in the final Sauvignon Blanc wines when SO<sub>2</sub> was added at around 120 mg/L at harvest [138]. However, the excessive use of SO<sub>2</sub> can have negative effects on human health, such as hives, swelling, headaches, stomach pain, and diarrhea [139]. In addition, the total SO<sub>2</sub> in wine is regulated and the use of SO<sub>2</sub> should be declared on the wine label in most winemaking countries. Therefore,

there is a trend that the use of SO<sub>2</sub> is minimized in wine production and the wine industry has always been seeking for alternatives to reduce the usage of SO<sub>2</sub>.

Common SO<sub>2</sub> alternatives used in wine production that have shown the efficacy of protecting juice and wine against oxidation are ascorbic acid and glutathione, to a lesser extent, glutathione-enriched inactivated dry yeast. Most of these alternatives have combined effects when used together with SO<sub>2</sub> while some of them need to be used complementary with SO<sub>2</sub>. Various combinations of these antioxidants have been studied to find the most effective and efficient way to protect the wines while keeping the SO<sub>2</sub> additions to a minimum level.

#### 4.1. Ascorbic Acid

Ascorbic acid has been utilized as an antioxidant with its capability to protect wines from oxidation by preferentially reacting with oxygen before the auto-oxidation of phenolic compounds happens and by reducing *o*-quinones back to the original phenolic compounds [103,140]. The oxidation of ascorbic acid generates hydrogen peroxide and dehydroascorbic acid, which is unstable and can degrade to a wide range of products [141]. Therefore, ascorbic acid is recommended to be used in conjunction with sulfur dioxide in wines as the presence of sulfur dioxide is essential to sufficiently remove hydrogen peroxide [142,143] and bind the dehydroascorbic acid and its degradation products [103].

Ascorbic acid protects white wines against oxidation and minimizes the browning of the wine under regular oxygen concentrations [144]. The combination of ascorbic acid and SO<sub>2</sub> better preserves fruity aromas as well as reduces oxidative aromas of the wines than using SO<sub>2</sub> alone [145]. Sauvignon Blanc wines supplemented with ascorbic acids and SO<sub>2</sub> were shown to contain higher levels of varietal thiols [146], and present higher intensities of fruity, grass, and green pepper aromas compared to wines added with SO<sub>2</sub> alone [147]. In addition, the presence of ascorbic acid decreases the requirement of SO<sub>2</sub> for a given amount of oxygen consumed in wines and thus extends the shelf-time of the wines [148,149]. However, in wines exposed to excessive amounts of oxygen either from poor bottling practices, inadequate closures, or long-term ageing, the role of ascorbic acid might convert from anti-oxidant to pro-oxidant [150,151]. This pro-oxidant activity relies on the rapid oxygen consumption of ascorbic acid together with SO<sub>2</sub>, which will result in browning and a shortened lifetime compared to ascorbic acid-free wines, and can even contribute to spoilage of the wine over a longer time [150]. Conversely, Sauvignon Blanc wines added with ascorbic acids but sealed with closures with low oxygen transmission rate were found to develop reductive characters after bottle ageing [52]. Therefore, TPO (total package oxygen) concentrations indicating the oxidation potential should be considered when bottling [150].

#### 4.2. Glutathione

Glutathione (GSH) is a tripeptide consisting of L-glutamate, L-cysteine, and glycine. The unique reducing and nucleophilic properties of glutathione that allow protein thiolation and modification of protein structure and function are ascribed to its free sulfhydryl moiety of the cysteine residue [152]. When acting as an antioxidant, glutathione can be oxidised enzymatically to glutathione disulfide (GSSG) [153]. Under unstressed conditions, GSSG can then be reduced back to glutathione by glutathione reductase, resulting in over 90% of glutathione existing in a reduced form [154,155].

Glutathione is naturally present in grapes and accumulates during grape maturation [156]. The concentration varies due to different grape varieties and is affected by both viticultural and oenological practices [107,114,157]. It can also be supplemented into musts or wine during the vinification process. A study revealed that glutathione added to Sauvignon Blanc juice shortly after crushing can provide antioxidant protection to the juice, reduce the use of SO<sub>2</sub>, and produce more volatile thiols in wines [158]. However, the amount of glutathione supplementation is regulated by the Organisation Internationale de la Vigne et du Vin (OIV) with a limited dose of no more than 20 mg/L in the must [159]. The

antioxidant properties of glutathione rely on the capability of its high affinity for oxygen which allows it preferentially oxidise its thiol group into a disulfuric group and form grape reaction products (2-S-glutathionyl tartaric acid), which terminates the oxidation process and thus protect other molecules from the attack of reactive oxygen species [160].

The effect of glutathione on the stability of wine flavour and wine colour has been demonstrated. It limits the formation of browning pigments by trapping *o*-quinones in a colourless form [161] and by limiting the production of xanthylium cation pigment precursors and *o*-quinone-derived phenolic compounds [162]. Additionally, glutathione protects varietal thiols from oxidation during bottle ageing [106], while its cysteinyl residue can be used as a source of sulfur to increase the concentration of polyfunctional mercaptans by reacting with trans-2-hexenal to form Glut-3MHal [163]. In addition, glutathione decreases the degradation of polyfunctional mercaptans during storage. Glutathione inhibits the decrease of several aromatic esters and terpene alcohols, such as isoamyl acetate, ethyl hexanoate, linalool, and  $\alpha$ -terpineol [164], as well as limits the accumulation of acetaldehyde [165] during storage. Furthermore, it suppresses the formation of stolon and 2-aminoacetophenone (2-AAP), which release unpleasant odours in wines and contribute to atypical wine ageing defects [166]. However, glutathione is found to favour the accumulation of hydrogen sulfide and methyl mercaptan, especially in the presence of copper under low oxygen conditions [167].

The use of glutathione in conjunction with SO<sub>2</sub> has a combined effect on protecting volatile thiols against oxidation [26], also, the addition of SO<sub>2</sub> slows down the enzymatic reduction of trans-2-hexenal and inhibits the enzymatic oxidative loss of glutathione [163]. The combination of glutathione and ascorbic acids strengthens the antioxidant capabilities and protects the phenolic compounds from oxidation [26], in addition, glutathione delays the loss of ascorbic acids and inhibits the reaction of ascorbic acids degradation products and (+)-catechin [162].

#### 4.3. Glutathione-Enriched Inactivated Dry Yeast

Despite glutathione has good antioxidant property, the application of glutathione to winemaking is still limited as the amount of glutathione supplementation is regulated [168]. The recommended approach of adding glutathione to juice or wine is through the addition of inactivated dry yeast (IDY) with guaranteed glutathione levels [169]. The preparations of glutathione-enriched inactive dry yeast (GSH-IDY) are manufactured from the thermal inactivation of *Saccharomyces cerevisiae*, which is cultivated under specific conditions (highly concentrated sugar medium) to stimulate the intracellular accumulation of glutathione. Commercial GSH-IDY were claimed to boost glutathione content either by liberating glutathione into the wine or by allowing the yeast to assimilate glutathione precursors during alcoholic fermentation for increased glutathione production [170]. Sauvignon Blanc wines supplemented with GSH-IDY preparations are shown to increase the concentration of certain volatile compounds, including thiols, higher alcohols, fatty acids, esters, and monoterpenes, and lead to higher intensities of aromas associated with riper tropical fruit than GSH-added wines [171]. The reasons that influenced the aroma profile of the wine are ascribed to the release of compounds other than glutathione by yeast products [26,171]. Previous studies that supplemented GSH-IDYs into the wines had some promising findings (Table 3), most of them affirming the antioxidant capability provided by glutathione released into the wine. More research is needed to better understand the full potential of this prospective antioxidant in white wine production, e.g., optimization of glutathione accumulation process, dosage rate, and timing of addition.

**Table 3.** Summary of previous studies on glutathione-enriched inactivated dry yeast (GSH-IDY).

Wine Matrix	Addition Timing	Key Findings	Reference
Sauvignon Blanc	Before fermentation	Increased thiols, higher alcohols, fatty acids, esters, and monoterpenes, leading to higher intensities of riper tropical fruity notes in wines.	[171]
Model wine	N.A.	Decreased the loss of typical wine terpenes.	[172]
Model wine	N.A.	Both yeast strain and glutathione accumulation process in preparation of GSH-IDY played an important role in the modulation of glutathione released into wine.	[173]
Model wine	N.A.	Yeast derivatives enriched with glutathione were more efficient at quenching radical species than those without glutathione enrichment.	[174]
Grenache Rosé	Before fermentation	More intense in fruity aromas (strawberry, banana) and less intense in yeast notes after 9 months ageing.	[175]
Sauvignon Blanc	After fermentation	Increased the release of polysaccharides into wines, and positive effects on the wine colour and on the prevention of wine oxidation.	[176]

## 5. Conclusions and Future Study

With low perception thresholds and distinctive aroma characters, methoxypyrazines and volatile thiols constitute the key varietal characteristics of Sauvignon Blanc wines. In addition to that, terpenes and other fermentative-derived volatile compounds are also important in adding complexity and imparting fruity notes to the wine. Being a fruit-driven wine dominated by primary aromas, one of the most important issues for Sauvignon Blanc wine production is oxidation during winemaking and ageing.

Methoxypyrazines are shown to remain stable after racking while oxidation condition including the additions of O<sub>2</sub>/SO<sub>2</sub> plays a minor role. However, inconsistent reviews present in the concentration changes during ageing. The discrepancy between the results is speculated to be the variables in the ranges of ageing periods, the grape varieties, or the ageing conditions, and thus further investigation is needed to confirm the influencing factors behind it. Compared to methoxypyrazines, volatile thiols are more prone to oxidation after alcoholic fermentation. They can either be oxidised into corresponding disulfides or be degraded to an odourless form. The oxidation mechanisms for volatile thiols are well studied, but more research is needed to build a comprehensive perspective of the concentration changes for 4MMP during ageing, as most studies only focused on the concentrations of 3MH and 3MHA. Additionally, further investigation is needed to understand the whole picture of biogenesis pathways and to fill the gap between the theoretical amounts of thiols backtracking from the precursors and the actual concentrations present in wine. During ageing, some monoterpenes are oxidized into corresponding terpene alcohol derivatives; esters concentrations decrease due to hydrolysis or oxidation; higher alcohols remain stable in concentrations as they can be oxidized to aldehydes but also be released from the oxidation of acids. The concentrations of certain volatiles such as aldehydes, lactones, and acetals increase, while some other volatile compounds evolve without a general trend during ageing.

In general, SO<sub>2</sub> is still the most commonly used antioxidant in wine production. Other SO<sub>2</sub> antioxidants, such as ascorbic acid and glutathione, have been commonly accepted to exhibit their antioxidant capabilities when added at harvest. Given that SO<sub>2</sub> has been confirmed to have negative effects on sulfite-sensitive individuals and can cause a detrimental effect on wine quality and thus should be limited in addition, finding antioxidants other than SO<sub>2</sub> to protect wines from oxidation during storage and transportation is especially crucial to the aromatic expression of New Zealand Sauvignon Blanc.

Inactivated dry yeasts enriched with glutathione with promising potential have been researched, and products have been developed and sold to commercial wineries. The suggested timing of supplement by the manufacturers is before alcoholic fermentation as the yeast derivatives are found to improve alcoholic fermentation and protect wines from oxidation at the early stage. However, previous experiments regarding GSH-IDYs were insufficient and the supplemented timing of GSH-IDYs was studied to a limited extent. Further investigation is needed to compare the difference between adding the glutathione-enriched yeasts before and after fermentation and to study the antioxidant capability of GSH-IDYs protecting wines during ageing.

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